Sources of Genetic Instability in Human Embryonic Stem Cells.

Grant Award Details

Sources of Genetic Instability in Human Embryonic Stem Cells.

Grant Type: SEED Grant

Grant Number: RS1-00428

Investigator:

Name:	Timothy O'Connor
Institution:	City of Hope, Beckman Research Institute
Type:	PI

Disease Focus: Cancer

Human Stem Cell Use: Embryonic Stem Cell

Award Value: $327,311

Status: Closed

Progress Reports

Reporting Period: Year 2

View Report

Grant Application Details

Application Title: Sources of Genetic Instability in Human Embryonic Stem Cells.

Public Abstract: The constant exposure of cells to endogenous and exogenous agents that inflict DNA damage requires active repair processes to eliminate potentially mutagenic events in stem cells leading to cancer. The same agents menace early human embryos with DNA damage that can ultimately lead to mutations, cancer, and birth defects. In vitro, human embryonic stem cells (HESCs) spontaneously undergo events leading to genetic instability and mutations. All these three types of genetic problems can have similar links to malfunctions in DNA repair systems, but little information now exists for HESCs. Therefore, the first step in understanding the causes of HESC genetic instability is to understand which DNA repair systems are defective. We will investigate the basis for this phenomenon in HESCs by evaluating their capacity to either repair DNA or form mutations. First, we will culture two HESC lines and compare HESC repair and mutation formation to that of control cells. We will use a new technique which simplifies the production and use of the feeder cells that support the growth of the HESCs. We will also test the genetic stability of HESCs grown on conventional feeder cells, as well as those grown in feeder free culture. We will use three types of DNA repair assays to monitor the genetic stability of the two HESC lines grown in these different ways. In the first of these assays, DNA molecules with different randomly-induced damage are transferred into HESCs, and DNA repair is followed by the re-establishment of the activity of a reporter protein that is coded for in the damaged DNA. A second assay will introduce specific DNA damage at a unique site in DNA that is transferred to HESCs and repair is determined using a polymerase chain reaction-based technique. Since aneuploidy is also known to be caused by double-strand DNA breaks, we will use two other assays to evaluate capacity of HESCs to repair that type of damage. These experiments will indicate if DNA repair pathways that eliminate DNA damage are dysfunctional and cause genetic instability. The final endpoint for these preliminary experiments is the formation of mutations. To study this, we have modified an assay system so that it will function in normal human cells to monitor mutations which arise spontaneously or those which are induced by various agents. In summary, these investigations will provide the basis for understanding genetic instability in HESCs that can direct cells to tumorgenic outcomes. The employment of HESCs clinically will require such knowledge. Moreover, these results will also yield information on susceptibility to mutations of cells early in development. The practical and basic science aspects of this seed grant proposal should lead to a complete proposal in the near future.

Statement of Benefit to California:	Human embryonic stem cells (HESCs) hold the potential to cure or alleviate many chronic illnesses, including cancer, but an immense gap exists between the achievement of the goals of stem cell based medicine and the current state of the art. Several stages of development including the following are required: (1) Routine, standardized, simple protocols for the indefinite growth of HESC in the normal, undifferentiated state, in completely defined medium.(2) Control over differentiation of the cells in (1) to all adult cell types of interest. (3) Control over the maintenance of the differentiated state of derivatives of (1), in sufficient complexity to recreate normal functional histology. (4) Techniques, therapies, and protocols that allow immune tolerance of regenerated tissue, without rendering the human recipient immunodefficient. Researchers are still struggling with steps 1 and 2. Although claims of feeder cell and animal product-free, long-term, undifferentiated HESC culture have been made, this is not the current state of the art in laboratories. These claims may be fortuitous or true for only a few HESC lines. The public anticipates a quick success of human stem cell technology and application to human disease, but the promise of stem cell therapy requires basic scientific work that is critical, but may not make headlines. Imprudent claims of miraculous cures could dim public enthusiasm. Few if any data exist regarding DNA repair systems or mutation frequencies of HESCs. We propose to investigate mechanisms underlying genetic instability in cultured HESCs. This instability limits HESC research and therapeutic applications. The data generated by this research according to Proposition 71 will be of lasting value to the People of the State of California for the following reasons: (1) This proposal focuses on a serious, basic difficulty with respect to the growth of undifferentiated HESCs that is a barrier to their human therapeutic use.(2) In the future, if the focus of the stem cell field shifts to the as yet unavailable somatic nuclear transfer (SNT) methods, this proposed research, will provide a basis for the comparison of HESCs and SNT cell lines. (3) All humans begin as embryonic stem cells, therefore data generated by the proposed research will impact maternal health, well baby programs, early childhood development/learning, etc, because mutations are involved in birth defects as well as cancer. Therefore, understanding the causes of mutations in HESCs could assist in avoidance or reduction in birth defects that would aid both the families and the government of California.(5) All of the work described in this proposal will be conducted by individuals in California and most probably will result in the hiring of a graduate of a California institution of higher education, thus reducing unemployment and helping educate a new generation of California researchers in HESC use.